Emirates Journal of Food and Agriculture. 2023. 35(1): 23-31
doi: 10.9755/ejfa.2023.v35.i1.2990
http://www.ejfa.me/

RESEARCH ARTICLE

The leavening ability of many lactic acid bacteria isolated from spontaneous sourdough

Youssef Mimoue Reffai[1]* and Mohammed idbella[2] and Toufik El Fechtali[1]

[1]Laboratory of Biosciences, Integrated and Molecular Functional Exploration, Faculty of Sciences and Techniques-Mohammedia, University Hassan II 146, Mohammedia 20650, Morocco, [2]Department of Agricultural Sciences, University of Naples Federico II, Via Universit 100, 80055 Portici, Italy

ABSTRACT

Sourdough technology has been used to make bread for thousands of years. While simple, it involves complex interactions that determine microbial communities. However, evidence for lactic acid bacteria-yeast interactions in sourdough is not yet sufficiently available. The interaction between lactic acid bacteria and yeast has been examined. This study aimed to isolate lactic acid bacteria in wheat sourdough and determine their functional properties, such as acidification kinetics, microbiological counts, exopolysaccharide (EPS) production, and leavening ability. The characterization of the isolates revealed the presence of 32 different strains. In addition, six strains with the best performance were chosen to be used as starters for monoculture and co-culture with baker's yeast. The lowest PH values and the highest total titratable acidity (TTA) values were observed with co-cultures. The growth rate analysis showed a negative interaction, whereas the lactic acid bacteria population was affected by yeast, while the yeast population size was rarely impacted, regardless of the strain. We found promising results for lactic acid bacteria strains regarding fermentation ability, which showed a higher ability to raise dough in monoculture. Two strains (2-1C2 and 13-1S2) showed interesting technological potential due to exopolysaccharide production and could be used to raise the dough without adding baker's yeast.

Key Words: Bread height, Baker's yeast, EPS production, Lactic acid bacteria, sourdough, leavening ability

INTRODUCTION

Sourdough is one of the oldest biotechnological processes used worldwide. Until the invention of Baker's Yeast, only the sourdough process could leaven bread (De Vuyst et al., 2014a; Meroth et al., 2003). A widely accepted definition describes sourdough as a mixture of flour and water fermented by action microorganisms, typically found in cereals and pseudo-cereals or inoculated as selected starters (De Vuyst et al., 2017a). This fermented dough contains a complex biological ecosystem where lactic acid bacteria (LAB) are the dominant organisms and coexist mainly with yeasts (Rocha & Malcata, 1999). In general, sourdough's LAB/yeast ratio varies from 100: 1 to 100:10 (De Vuyst & Neysens, 2005a). It has been found that sourdough contributions can enhance the nutritional value and sensory properties of bakery goods, extend shelf life, delay staling, and reduce or partially replace the use of additives in bakery goods, which has attracted the attention of consumers (Dong & Karboune, 2021; Rizzello et al., 2014; Scarnato et al., 2017). These characteristics are mainly linked to sourdough micro-flora, particularly lactic acid bacteria (Corsetti & Settanni, 2007a). Nevertheless, the endogenous factors of cereal products (carbohydrates, sources of nitrogen, minerals) and process parameters (temperature, fermentation time, dough yield, oxygen, and the number of leaven propagation stages) strongly influence the sourdough micro-flora (De Vuyst et al., 2014b).

Leavening is the slowest and most rate-limiting step in any baking process, mainly when it relies on only endogenous LAB and yeast (De Vuyst et al., 2017b). In most cases, LABs are responsible for most of the dough acidification process, whereas yeast is largely responsible for increasing dough volume through carbon dioxide production (Valmorri et al., 2010). In sourdough, where yeast accounts for about 1% of the total number of cells, it has been suggested that yeast provides approximately 15-20% of the total CO2 formed, with the remainder supplied by lactic acid bacteria strains (Hammes & Gänzle, 1998a). The interactions between

***Corresponding author:**
Youssef Mimoue Reffai, Laboratory of Biosciences, Integrated and Molecular Functional Exploration, Faculty of Sciences and Techniques-Mohammedia, University Hassan II 146, Mohammedia 20650, Morocco.**E-mail**: refm.youssef@gmail.com

Received: 14 August 2022; **Accepted:** 30 December 2022

lactic acid bacteria and yeast must be considered to achieve a balanced metabolism. Therefore, sourdough is considered one of the most effective methods for enhancing bread volume without relying on additives(Fraberger et al., 2020).

Nowadays, the use of traditional sourdough as a leavening agent has decreased because of commercial yeast's convenience. Consequently, the positive aspects of sourdough bread were overlooked (Sakandar et al., 2019). To compensate for the main benefits of traditional sourdough, the modern biotechnology of baking often uses a commercial starter culture that includes both LABs and yeasts. The selection criteria for starter cultures differ according to the attributes targeted (i.e., technological, biochemical, and nutritional characteristics. Acidification and growth rates are the most frequently assessed criteria for selection, as they are the root cause of all the changes during fermentation (Paramithiotis et al., 2005).

This research aims to isolate LABs from the microbiota of traditionally sourdough and determine their functional properties, such as acidification, growth rates, EPS production, and Gaz production(CO2), as well as to investigate interactions between S. cerevisiae and the LAB to observe their influence on specific characteristics and to evaluate their suitability as starters or adjunct cultures.

MATERIALS AND METHODS

Preparation of mature sourdough

A laboratory sourdough was made to isolate LAB. The mature sourdough was prepared by mixing 50g of commercial wheat flour with 50 mL of tap water (1:1 w/v). The ingredients were mixed for 5 min until a thick, homogeneous batter was formed, then left to ferment for 24h at (30 C). The next day, the dough was kneaded after mixed with freshly batch 10 g of flour and water (30 °C). This procedure was repeated for three days to keep dominating microorganisms in an active state. At the end of the third day, the dough was fully fermented and formed many holes in its mass and surface, which indicate vigorous fermentation activity.

Isolation and counting of LAB

Two samples of the mature sourdough were coded 1 and 2 analyzed by taking samples from the surface (S1 & S2) and the core (C1 & C2), while the rest were stored at 4C. PH and TTA were assessed according to a standard method (Meroth et al., 2003). For the enumeration, Lactic Acid Bacteria (LAB) were cultivated in modified De Man, Rogosa and Sharp medium (MRS) modified according to (Ricciardi et al., 2015). The culture medium was supplemented with cycloheximide to prevent the growth of yeasts and molds. Counts were performed following the spread-plate method according to (Reale et al., 2011). The results are expressed as a log of colony-forming units (CFUs) per gram of sample. We measured each model twice and calculated the mean values of pH, TTA, and (CFUs).

LABs purification and pre-identification

Several Lactic acid bacteria isolates were identified using morphological and physiological studies. Different types of colonies from MRS counting plates were selectively counted and tentatively classified by cell observation at the microscope. Plates seeded with the highest dilutions to assess the dominant lactic micro-flora obtained 32 LAB cultures (12 from the first and 20 from the second). Each colony was purified by repeated streaking on MRS agar after incubation for 48 h at 30°C. All isolates were stored at 4°C in YDA agar.

RAPD-PCR genotypic characterization

RAPD analyzed the isolated LAB strains- PCR (Random Amplified Polymorphic DNA) using the primer M13-R2 (5'-GAG GGT GGC GGT TCT -3'). The DNA extraction was performed according to (Aponte et al., 2012). The Gels were analyzed using the bio-numerics software version 5.1 (Applied Maths, Kortrijk, Belgium). RAPD-PCR patterns were grouped using cluster analysis with Pearson's product-moment correlation coefficient and the unweighted pair group method using arithmetic averages.

LAB strains' technological and biochemical characterization

For the identification of potential starter cultures, 32 presumptive LABs were selected and analyzed based on the following criteria:

Acid fermentation: The cultures were tested for their ability to ferment arabinose, fructose, maltose, starch, and maltose plus fructose. Strains were grown in MRS medium (pH 7.2-7.8) without glucose and Lab-lemco, supplemented with phenol red (0.17 g/L), and filtered sterilized sugar solutions (0.5 vol/vol). We evaluated the ability to ferment starch using drop-inoculated starch agar plates. Plates were incubated at 30°C until colonies were visible and then covered with Lugol solution (iodine solution) starch indicator for 1 min. Strains were considered positive if they had a clear halo around them.

EPS: The ability to produce exopolysaccharides (EPS) was tested in ATP medium (Difco) added of ruthenium red (0.08g/L) and supplemented with filter-sterilized solutions (1% v/v) of 17 different sources of carbohydrates.

Leaving ability test: Gaz and acid production.

Commercially available semolina flour is used for dough preparation. 15g of semolina flour was added to the microbial suspension and mixed manually until a good dough developed. We fermented the dough for 16 h at 30°C. We determined the leavening ability of the different strains by measuring the height reached by the dough after 16 h of fermentation at 30°C. A short meter rule is placed, and the level is read. The number of viable LABs was determined before and after the leavening using the drop count method (Collins, 2004). A sample of the dough was diluted up to 10-9. 12 µl aliquots were spotted onto MRS agar plates. After incubation for 24 h at 30°C, colonies in each countable drop were counted. We determined TTA and pH at time zero and after incubation for each strain. Control samples were prepared as follows: Straight dough sample (Control 1: wheat (semolina) flour + Ringer solution), (Control 2: semolina flour + RS + baker's yeast).

Analysis of data

All statistical analyses were performed using SPSS23.0 software, and all graphs were prepared using Excel software for Windows version 7. We used a one-way analysis of variance (ANOVA) to determine the differences between the means. The correlation analysis was performed using bivariate correlations according to Pearson. A P level of 0.01 was considered significant.

RESULTS AND DISCUSSION

Strain-typing by M13 RAPD-PCR

The dendrogram of LAB strains is shown in Fig. 1. 32 LAB strains were isolated from traditionally sourdough that belonged to the microbiota on the surface and core. Furthermore, the genotypes were characterized using RAPD PCR, and several of the tested strains showed the same RAPD profile. The total number of profiles was thus reduced to 18.

To assess this ecosystem microbial diversity, the thirty-two isolated strains were used for further analyses (acidity, growth rate, leavening ability, acid fermentation, and EPS production) to develop a new starter culture that may be very promising for industries with special needs for microorganisms at various capacities.

Biochemical characterization of the isolates LAB strains

i. Ability to ferment carbohydrates

The LAB isolates were tested for their ability to ferment maltose, Arabinose, fructose, and the maltose + fructose mixture, which are the main soluble carbohydrates in sourdough. The results showed that all strains were able to ferment all the sugars provided. These highly adaptive to different sources of carbohydrates could indicate the strains' diversity and reduce metabolic competition with yeasts. Besides, this feature is essential for those that will be used later in the dough-making process. Based on carbohydrates' metabolic pathways, lactic acid bacteria are classified into facultative and obligate hetero-fermentative, and obligate homo-fermentative. Nevertheless, it should be emphasized that the metabolic pathways for carbohydrates can be changed by the same microbial strain throughout time, depending on the environment and growth conditions (Siezen et al., 2002). The traditional dough is dominated by hetero-fermentative species, which are considered decisive in sourdough type I, mainly explained by their adaptation to this specific environment (Corsetti & Settanni, 2007b; De Vuyst & Neysens, 2005b). Furthermore, our findings support the presence of both homo and hetero-fermentative lactic acid bacteria, the latter being more predominant. In contrast, none of the strains tested was able to express starch hydrolysis. As a result, these strains may have lacked this property or need further analysis to identify this feature that influences bread quality (Singh et al., 2015).

ii. EPS production test

Table 1 shows EPS production from LAB isolated. A total of 17 carbon sources were used to evaluate EPS biosynthesis (Table 1). It must be pointed out that the strain (2-1C2) could produce EPS in the presence of all the carbon sources provided for it, including the use of starch and pentose. In addition, two strains (13-1C2 and 6-1C2) showed the EPS production activity of 16 from 17 carbon sources, except starch and Rhamnose, respectively. The LAB's diversity has been emphasized by more than 54% of strains (17 out of 32) producing EPS from ten different carbon sources, suggesting that these strains have at least one gene required for EPS-forming (Ispirli et al., 2018). Furthermore, several studies have reported that the micro-flora of traditionally prepared sourdough usually consists of two to five strains that produce EPS (Tieking & Gänzle, 2005). Moreover, EPS production significantly affects different properties, such as increasing dough viscoelasticity, bread volume, reducing bread hardness, and extending shelf life (Poutanen et al., 2009; Yıldız et al., 2019). Additionally, it has been suggested that EPS in a sourdough environment could have prebiotic effects (Dertli et al., 2015). That property can satisfy consumers' demand by reducing food additives.

Technological characterizations of the isolated LAB strains

All the strains were evaluated for their fermentation abilities in a system imitating sourdough fermentation conditions.

i. Acidity and Microbial counts

Table 2 shows the PH values, total titratable acidity (TTA), and cell viability after 16 h of fermentation. It has been

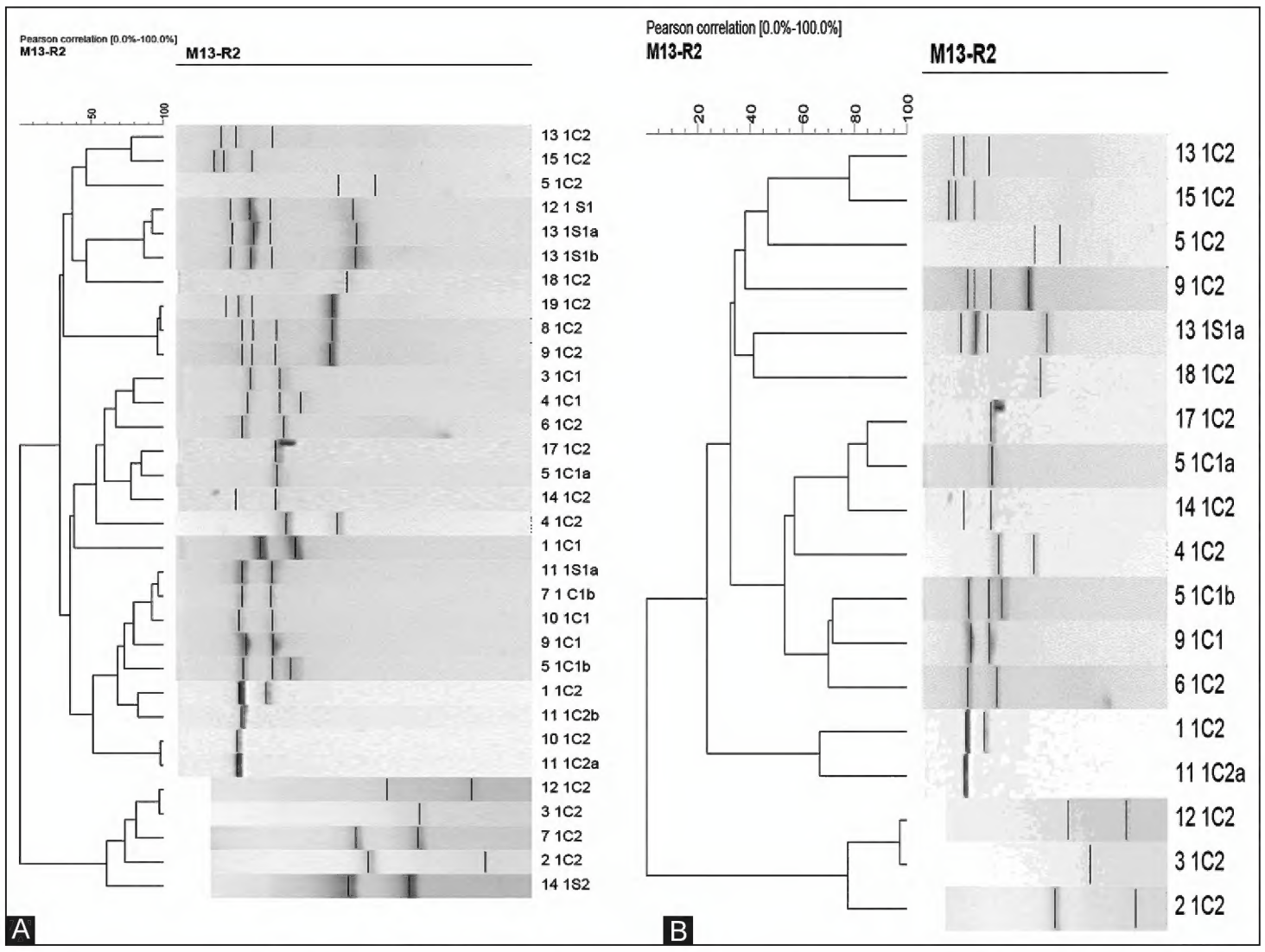

Fig 1. (A-B) The Dendrogram obtained from M13 RAPD-PCR fingerprints of the LAB strains isolated from the sourdough.

noticed that the pH before fermentation is similar for all LAB strains; it varies from 5.18 to 6.3. However, the pH values are slightly lower than the control (pH = 6.36). From the table, we can see a considerable variation (decrease) in the pH after fermentation for all the LAB strains; it varied between 3.45 and 3.9, except for the strains 2-1C2, 15-1S2, 13-1S1, 9-1C2, and 4-1C2, which presented an average pH after fermentation of 4.89, 4.81, 4.68, 4.36 and 4.28, respectively. This variation in pH is high compared to the control that shows a pH of 4.94 after fermentation. A TTA (Table 2) was determined to provide additional information about fermentation metabolism and acidity, and it was correlated linearly with pH (r = −0.704; p=0.01) since a decrease in pH was consistent with an increase in the mL of NaOH/10 g of dough. The results showed that the sourdough sample produced in this study exhibited pH and TTA evolution typical of traditional sourdoughs. Additionally, most strains showed low growth (table 2), except five strains (4-1C2, 1-1C2, 2-1C2,13-1S1, and 15-1S2) which registered the highest concentration of viable cells after 16 hours of fermentation, which reached 8.1 CFU/g, 6.9 CFU/g, 6 CFU/g, 4.6 CFU/g, and 4.2 UFC/g respectively. In addition, three strains (18-1S2, 3-1C2, and 13-1S2) showed results comparable to the control.

The results showed that most strains could reduce the pH values to less than 4 after 16 hours of fermentation, affecting growth and metabolic activity (Gobbetti et al., 2005). In this study, we observed that LAB's response to the acidification change varies according to the adaptation of the strains in the environment. In addition, the difference in nutrient supply between the imitating medium and flour may affect microbes' responses (Hammes & Gänzle, 1998b). After 16 hours of fermentation, LAB CFUs were related to dough pH. There was a significant correlation (p = + 0.534, significant at 0.01). The significant drop in pH values is caused by an increase in acids (i.e. lactic acid and acetic acid), and the accumulation of these major end-products in the medium can influence bacterial growth and act as a factor limiting the growth of lactic acid bacteria. (Hammes & Gänzle, 1998b) reported that lactobacilli do not grow well at a pH below 3.8, and the optimum pH is between 4.2 and 6.4 depending on the species. Moreover, the strains (2-1C2, 13-1S2, 4-1C2, and 1-1C2) presented average values for reducing the PH and were better adapted to the acid environment, leading to the best growth rate.

Height

The capacity of the different LABs to raise the dough was assessed by measuring the height (cm) reached by the dough samples after 16h of fermentation (Table 2). It has been noticed that most strains' performance is deficient compared to the control (semolina + Ringer solution). Nonetheless, strains 13-1S(1), 12-1S1, 13-1S(2), 2-1C2 have shown a higher ability to raise the dough than the control (2.5cm), which reached values of 5.1 cm, 4.7 cm, 4.6 cm, and 4.4 cm, respectively. These results consider the enormous ability to produce CO2, indicating that these strains are obligatory hetero-fermentative lactic acid bacteria. For some strains, such as 4-1C1, 5-1C2, 8-1C2,

Table 1 : Biochemical characterization of LAB strains isolated from sourdough: EPS production test

	Arabinose	Cellobiose	Dextrose	Fructose	Galactose	Lactose	Lyxose	Maltose	Mannose
13-1C2	+	+	+	+	+	+	+	+	+
15-1C2	-	-	-	-	-	-	-	-	-
5-1C2	-	-	-	-	-	-	-	-	-
9-1C2/8-1C2	-	-	-	-	-	-	-	-	-
19-1C2	+	+	+	+	+	+	+	+	+
13-1S1a	+	+	+	+	+	+	-	+	+
13-1S1b	+	+	+	+	+	+	-	+	+
12-1S1	+	+	+	+	+	+	-	+	+
18-1C2	-	-	-	-	-	-	-	-	-
17-1C2	-	-	-	-	-	-	-	-	-
5-1C1a	-	-	-	-	-	-	-	-	-
14-1C2	-	-	-	-	-	-	-	-	-
7-1C2	+	+	+	+	+	+	-	+	+
4-1C2	-	-	-	-	-	-	-	-	-
5-1C1b	+	-	+	+	+	+	-	+	-
4-1C1	+	-	+	+	+	+	-	+	-
1-1C1/3-1C1	+	+	+	+	+	+	-	+	-
7-1C1(b)/9-1C1/10-1C1	+	+	+	+	+	+	-	+	+
11-1C1 (a)	+	+	+	+	+	+	-	+	-
6-1C2	+	+	+	+	+	+	+	+	+
1-1C2	-	-	-	-	-	-	-	-	-
11-1C2a/11-1C2b	-	-	-	-	-	-	-	-	-
10-1C2	+	-	+	+	+	+	+	+	+
12-1C2	-	-	-	-	-	-	-	-	-
3-1C2	-	-	-	-	-	-	-	-	-
2-1C2	+	+	+	+	+	+	+	+	+

	Melibiose	Melezitose	Raffinose	Rhamnose	Starch	Sucrose	Trehalose	Xylose
13-1C2	+	+	+	-	+	+	+	+
15-1C2	-	-	-	-	-	-	+	-
5-1C2	-	-	-	-	-	-	-	-
9-1C2/8-1C2	-	-	-	-	-	-	-	-
19-1C2	+	+	+	-	+	-	+	+
13-1S1a	+	+	-	-	-	+	-	+
13-1S1b	+	+	-	-	-	-	-	+
12-1S1	+	+	+	-	-	+	-	+
18-1C2	-	-	-	-	-	-	-	-
17-1C2	-	-	-	+	-	-	-	-
5-1C1a	-	-	-	-	-	-	-	-
14-1C2	-	-	-	-	-	-	-	-
7-1C2	+	+	+	-	-	+	-	+
4-1C2	-	-	-	-	-	-	-	-
5-1C1b	+	+	-	-	-	+	-	+
4-1C1	+	+	-	-	-	+	-	+
1-1C1/3-1C1	+	+	-	-	-	+	-	+
7-1C1(b)/9-1C1/10-1C1	+	+	+	-	-	+	-	+
11-1C1 (a)	+	+	-	-	-	-	-	+
6-1C2	+	+	+	+	+	-	+	+
1-1C2	-	-	-	-	-	-	-	-
11-1C2a/11-1C2b	-	-	-	-	-	-	-	-
10-1C2	+	+	+	-	+	-	+	+
12-1C2	-	-	-	-	-	-	-	-
3-1C2	-	-	-	-	-	-	-	-
2-1C2	+	+	+	+	+	+	+	+

and 12-1C2, the CO2 production property was absent, and they acted as homofermentative LABs. It is clear from these differences that the effect of LAB strains on dough height differs from strain to strain.

Table 2: Height, pH, TTA, values and Bacterial count for the isolated strains after 16h of fermentation

Strains Code	pH	TTA	Growth	Height (cm)
1-1C1	3.79	12.50	0.50	0.60
1-1C2	3.81	13.00	2.5	0.60
10-1C1	3.54	13.2	0.80	0.70
10-1C2	3.62	14.5	-0.60	0.80
11-1C1	3.65	12.5	1.05	1.03
11-1C2	3.76	9.00	0.25	0.60
11-1S1	3.45	18.2	0.75	0.65
11-1S2	3.82	13.9	-1.00	0.40
12-1C2	4.16	6.89	1.22	0.00
12-1S1	3.64	16.50	0.30	4.70
13-1C2	3.90	11.2	1.37	1.02
13-1S1	3.58	15.00	4.60	5.10
13-1S2	4.68	13.80	3.10	4.60
14-1C2	3.54	15.75	1.00	0.75
15-1S2	4.81	14.80	4.20	2.70
17-1S2	3.49	16.00	1.00	0.90
18-1S2	4.05	11	2.47	2.50
19-1S2	3.77	14.25	-1.10	0.30
2-1C2	4.89	5.50	6.00	4.40
3-1C1	nd	11.00	0.20	0.30
3-1C2	3.72	15.00	2.8	4.40
4-1C1	3.74	16.00	0.50	0.00
4-1C2	4.28	13.00	8.10	0.60
5-1C1	3.83	15.8	0.60	0.35
5-1C2	3.85	10.5	0.9	00
5-1S2	3.5	17.5	1.1	0.90
6-1C2	3.69	13.7	0.30	0.20
7-1C1	3.89	14.00	0.4	0.25
7-1C2	3.87	15.60	-0.10	0.30
8-1C2	nd	14.50	-0.20	0.00
9-1C1	3.52	12.9	0.20	0.40
9-1C2	4.36	8.50	-0.70	0.60
Control	5.46	2.50	2.50	2.80
Mean±S.D.	3.87±0.37	13.4±3.1	1.45±2.23	1. 3±1.5

S.D.: standard deviation. (Values are mean±standard deviation of 2 replicated assays).
n.d.: not determined.

Combination LAB-Baker's yeast

The strains with the best performance in terms of leavening ability were selected to be employed as potential starters for sourdough production. A slight difference in pH and TTA in monoculture and co-culture was noticeable, except for the strain (2-1C2), which showed high acid production in the presence of baker's yeast (Fig.s 2A and 2B). However, the LAB/BY association performs better in acidity production. The lowest PH values and the highest TTA values were observed with the co-cultures. In this regard, the results of (Paramithiotis et al., 2006) are very similar since they also note a slight difference between monoculture and co-culture.

The microbial growth of LAB (Fig. 2C) seemed to be negatively affected when baker's yeast was present, as the co-cultures (13-1S1/BY, 13-1S2/BY, and 12-1S1) resulted in a significant decrease in the growth rates of LABs, with slight or neutral changes for the strains (12-1S/BY and 15-1S2/BY). The considerable decrease observed in the yeast's presence might be attributed to the competition for available carbohydrates since the S. cerevisiae (BY) strain prefers maltose, which may negatively impact the lactic acid bacteria's behaviour and growth rate (De Vuyst et al., 2014). The faster consumption of maltose and, in particular, glucose by the yeast (maltose positive) is responsible for forming unstable and competitive associations (De Vuyst et al., 2009; Gobbetti et al., 1995). Consequently, yeasts and LAB produce and release compounds that alter physicochemical properties in positive or negative ways in the dough environment (Arora et al., 2021).

The yeast's growth rate (2D) was unaffected by the presence of lactic acid bacteria, and their adaptation explains high yeasts' tolerance to acidic environments and their resistance to nutrient competition. Furthermore, our results align with other findings that found the same observation about synergistic and mutuality effects between LABs and yeasts (Carbonetto et al., 2020; Häggman & Salovaara, 2008; Paramithiotis et al., 2006). The specific cooperation (cross-feeding) between yeast and LAB is required for metabolic activity balance and improved sourdough stability. Stable associations also develop among species that can utilise a variety of carbohydrates, such as between the common sourdough species L. Plantarum, L. sanfransiscensis and S. cerevisiae (De Vuyst et al., 2009).

In contrast, the co-culture (15-1s2+BY) showed a decrease in yeast microbial load, probably due to the reasons above, which are not related to acidity (no significant correlation detected), but to the negative interactions between LAB and yeast, because some strains produce high acidity, such as the strain 13-1S1, it did not affect yeast growth, and it grew well in its presence compared to the control.

Leavening is one reason for fermentation in the baking process. To leaven dough, gas formation by the micro-flora is required. In sourdough, yeast and hetero-fermentative LAB produce CO2, and each group's participation in the overall gas volume depends on the type of starter and technology applied (Lim et al., 2018). Therefore, we compared the maximum gas retention (Fig. 2E) between mono and co-cultures of the same Baker's yeast with different LAB strains by measuring the height reached after 16 hours of fermentation. Among the combinations, promising results were obtained using strains 13-1S2+BY and 2-1C2+BY, in which the leavening after 16 hours was 4.9 cm and 4.7cm, respectively. These two associations showed very high performances compared to control 1 (semolina + RS) and slightly superior to control 2 (containing Baker's yeast). Furthermore, the co-cultures

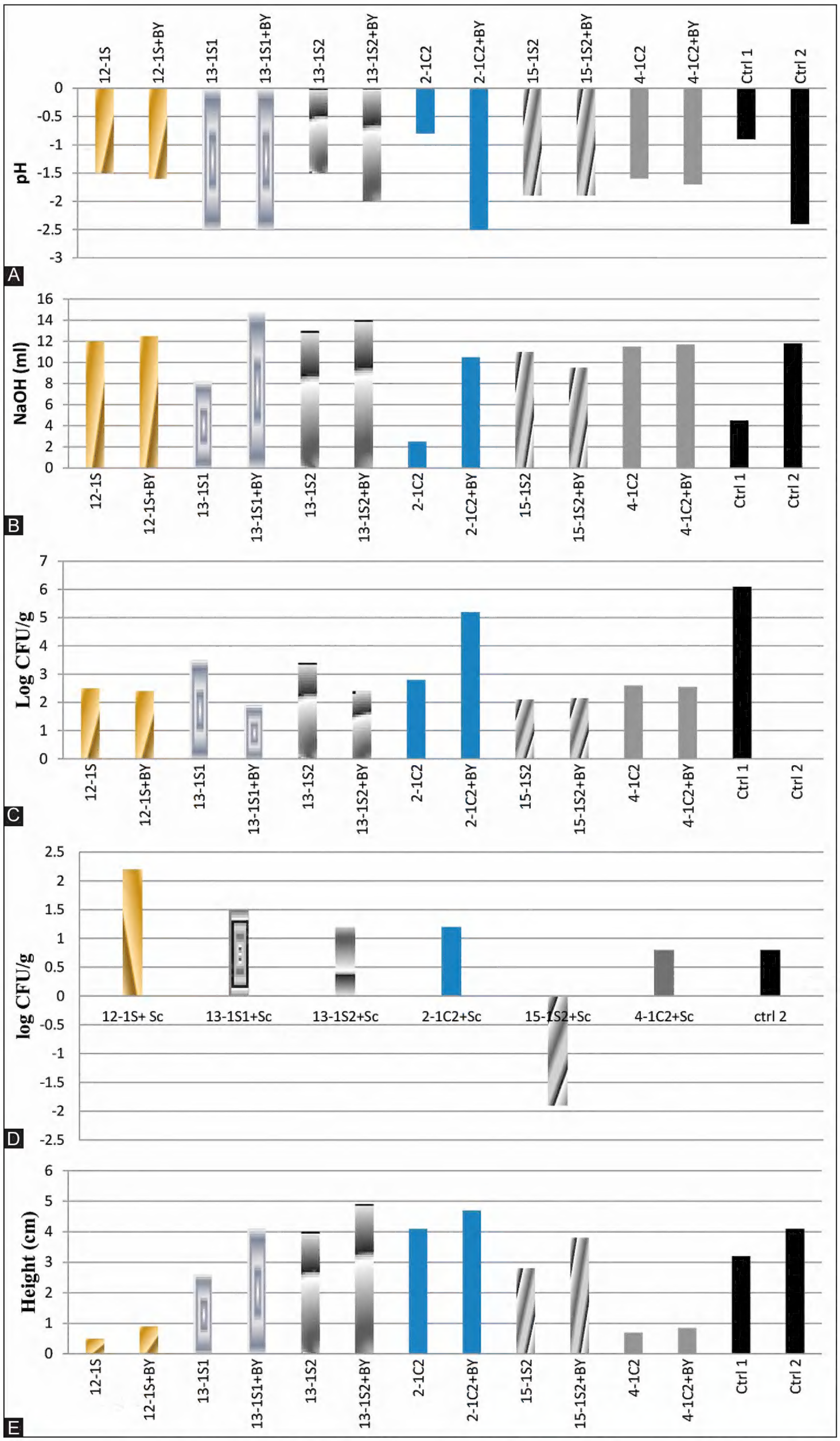

Fig 2. (A) pH values in monoculture and co-cultures after 16h of fermentation. (B) TTA values in monoculture and co-cultures after 16h of fermentation. (C) LAB growth values in monoculture and co-cultures after 16h of fermentation. (D) yeast growth values in co-cultures after 16h of fermentation. (E) Height values in monoculture and co-cultures after 16h of fermentation.

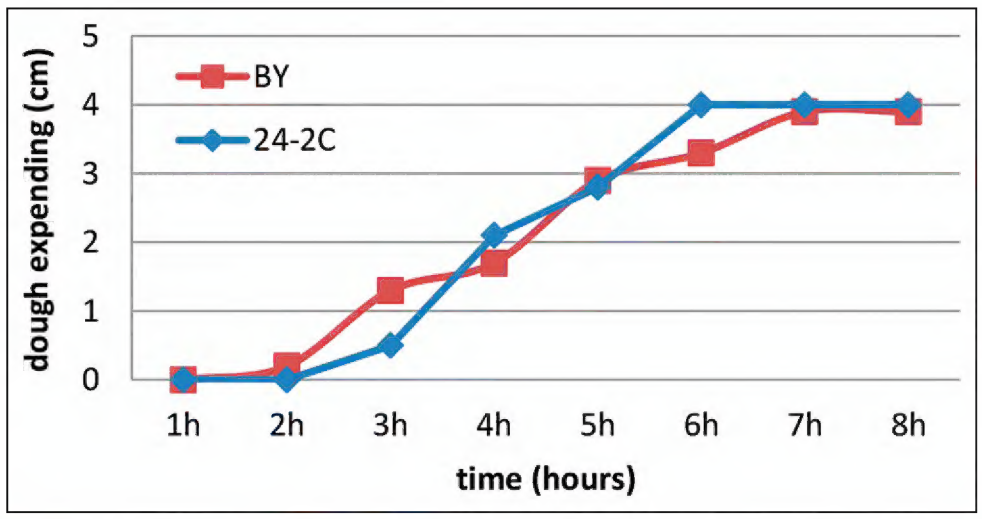

Fig 3. Monitoring of dough expansion as a function of time for the strain 2-1C2 and the baker's yeast.

13-1S1/BY and 15-1S2/BY achieved similar results to the Baker's yeast alone.

In contrast, the other two strains (12-1S/BY, 4-1C2/BY) showed a lower ability to raise the dough. In all cases, when the six strains were added, the results revealed that the LAB/yeast association outperformed the levitation carried out by the LAB alone. It has been found that yeast provides about 15-20% of the total CO2 produced in sourdough; the remainder is provided by lactic acid bacteria.

To obtain additional information on the CO2 production ability, a comparison was made between the fermentation capacities of the isolated strain LAB (2-1C2) and commercial Baker's. The chart below (Fig. 3) shows the amount of dough expansion overtime for the strain 2-1C2 and the Baker's yeast. A similar performance has been noticed between the two strains. However, strain 2-1C2 performed slightly faster than the Baker's yeast: It reached a maximal value of 4 cm after 6 hours, while the Baker's yeast reached its maximal value (3.9 cm) after 7h. Accordingly, the dough prepared traditionally is sufficient to achieve the appropriate height for bread in less time than Baker's yeast. Furthermore, previous studies state that the existence of the right LAB strain in the sourdough is sufficient to ensure the leavening of bread without using Baker's yeast and may provide many other advantages (Häggman & Salovaara, 2008; Hammes & Gänzle, 1998b; Li et al., 2014).

CONCLUSION

Traditional sourdough samples were submitted for physicochemical and microbiological characterizations. The combination of analysis of biochemical (EPS production and carbohydrate fermentation tests) and molecular evidence (RAPD- PCR) means that 26 different strain profiles have been demonstrated. These should be identified by 16S rDNA partial sequencing. 32 LAB strains were isolated, and following technological selection tests, in particular leavening ability tests, six strains proved to be promising. These have shown a leavening capability comparable or better than conventional baker's yeast. The dough prepared with the combination of these strains and baker's yeast showed even higher levitation levels compared to the control (Baker's yeast alone). Sourdough has undoubted advantages over any other leavening agent in terms of rising dough.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest. The authors alone are responsible for the content and writing of this article.

Authors' contribution

Toufik El Fechtali: Conceptualization, Resources, review and editing, Supervision. Mohammed idbella: designed and performed the experiment and supervision. Youssef Mimoue Reffai: performed the experiments and analyzed the data and wrote the manuscript. All authors have read and approved the final manuscript to be published

REFERENCES

Aponte, M., G. Blaiotta, F. La Croce, A. Mazzaglia, V. Farina, L. Settanni and G. Moschetti. 2012. Use of selected autochthonous lactic acid bacteria for Spanish-style table olive fermentation. Food Microbiol. 30: 8-16.

Arora, K., H. Ameur, A. Polo, R. Di Cagno, C. G. Rizzello and M. Gobbetti. 2021. Thirty years of knowledge on sourdough fermentation: A systematic review. Trends Food Sci. Technol. 108: 71-83.

Carbonetto, B., T. Nidelet, S. Guezenec, M. Perez, D. Segond and D. Sicard. 2020. Interactions between *Kazachstania humilis* yeast species and lactic acid bacteria in sourdough. Microorganisms. 8: 240.

Corsetti, A. and L. Settanni. 2007a. Lactobacilli in sourdough fermentation. Food Res. Int. 40: 539-558.

Corsetti, A. and L. Settanni. 2007b. Lactobacilli in sourdough fermentation. Food Res. Int. 40: 539-558.

De Vuyst, L. and P. Neysens. 2005a. The sourdough microflora: Biodiversity and metabolic interactions. Trends Food Sci. Technol. 16: 43-56.

De Vuyst, L. and P. Neysens. 2005b. The sourdough microflora: Biodiversity and metabolic interactions. Trends Food Sci. Technol. 16: 43-56.

De Vuyst, L., S. Van Kerrebroeck, H. Harth, G. Huys, H. M. Daniel and S. Weckx. 2014a. Microbial ecology of sourdough fermentations: Diverse or uniform? Food Microbiol. 37: 11-29.

De Vuyst, L., S. Van Kerrebroeck, H. Harth, G. Huys, H. M. Daniel and S. Weckx. 2014b. Microbial ecology of sourdough fermentations: Diverse or uniform? Food Microbiology, 37, 11-29.

De Vuyst, L., S. Van Kerrebroeck and F. Leroy. 2017a. Microbial ecology and process technology of sourdough fermentation. In: Advances in Applied Microbiology. Vol. 100. Elsevier, Amsterdam, Netherlands, pp. 49-160.

De Vuyst, L., S. Van Kerrebroeck. and F. Leroy. 2017b. Microbial

ecology and process technology of sourdough fermentation. In: Advances in Applied Microbiology. Vol. 100. Elsevier, Amsterdam, Netherlands, pp. 49-160.

De Vuyst, L., G. Vrancken, F. Ravyts, T. Rimaux and S. Weckx. 2009. Biodiversity, ecological determinants and metabolic exploitation of sourdough microbiota. Food Microbiol. 26: 666-675.

Dertli, E., M. J. Mayer and A. Narbad. 2015. Impact of the exopolysaccharide layer on biofilms, adhesion and resistance to stress in *Lactobacillus johnsonii* FI9785. BMC Microbiol. 15: 8.

Dong, Y. and S. Karboune. 2021. A review of bread qualities and current strategies for bread bioprotection: Flavor, sensory, rheological, and textural attributes. Comprehens. Rev. Food Sci. Food Saf. 20: 1937-1981.

Fraberger, V., C. Ammer and K. J. Domig. 2020. Functional properties and sustainability improvement of sourdough bread by lactic acid bacteria. Microorganisms. 8: 1895.

Gobbetti, M., A. Corsetti and J. Rossi. 1995. Interaction between lactic acid bacteria and yeasts in sour-dough using a rheofermentometer. World J. Microbiol. Biotechnol. 11: 625-630.

Gobbetti, M., M. De Angelis, A. Corsetti and R. Di Cagno. 2005. Biochemistry and physiology of sourdough lactic acid bacteria. Trends Food Sci. Technol. 16: 57-69.

Häggman, M., H. Salovaara. 2008. Microbial re-inoculation reveals differences in the leavening power of sourdough yeast strains. LWT. Food Sci. Technol. 41: 148-154.

Hammes, W. P. and M. G. Gänzle. 1998a. Sourdough breads and related products. In: B. J. B. Wood (Ed.), Microbiology of Fermented Foods. Springer US, New York City, pp. 199-216.

Hammes, W. P. and M. G. Gänzle. 1998b. Sourdough breads and related products. In: B. J. B. Wood (Ed.), Microbiology of Fermented Foods. Springer US, New York City, pp. 199-216.

Ispirli, H., F. Demirbaş, M. O. Yüzer and E. Dertli. 2018. Identification of lactic acid bacteria from spontaneous rye sourdough and determination of their functional characteristics. Food Biotechnol. 32: 222-235.

Li, W., J. Ji, X. Rui, J. Yu, W. Tang, X. Chen, M. Jiang and M. Dong. 2014. Production of exopolysaccharides by *Lactobacillus helveticus* MB2-1 and its functional characteristics *in vitro*. LWT. Food Sci. Technol. 59: 732-739.

Lim, S. B., J. M. R. Tingirikari, J. S. Seo, L. Li, S. Shim, J. H. Seo and N. S. Han. 2018. Isolation of lactic acid bacteria starters from Jeung-pyun for sourdough fermentation. Food Sci. Biotechnol. 27: 73-78.

Meroth, C. B., J. Walter, C. Hertel, M. J. Brandt and W. P. Hammes. 2003. Monitoring the bacterial population dynamics in sourdough fermentation processes by using PCR-denaturing gradient gel electrophoresis. Appl. Environ. Microbiol. 69: 475-482.

Paramithiotis, S., Y. Chouliaras, E. Tsakalidou and G. Kalantzopoulos. 2005. Application of selected starter cultures for the production of wheat sourdough bread using a traditional three-stage procedure. Process Biochem. 40: 2813-2819.

Paramithiotis, S., S. Gioulatos, E. Tsakalidou and G. Kalantzopoulos. 2006. Interactions between *Saccharomyces cerevisiae* and lactic acid bacteria in sourdough. Process Biochem. 41: 2429-2433.

Poutanen, K., L. Flander and K. Katina. 2009. Sourdough and cereal fermentation in a nutritional perspective. Food Microbiol. 26: 693-699.

Reale, A., T. Di Renzo, M. Succi, P. Tremonte, R. Coppola and E. Sorrentino. 2011. Identification of lactobacilli isolated in traditional ripe wheat sourdoughs by using molecular methods. World J. Microbiol. Biotechnol. 27: 237-244.

Ricciardi, A., A. Guidone, R. G. Ianniello, S. Cioffi, M. Aponte, D. Pavlidis, E. Tsakalidou, T. Zotta and E. Parente. 2015. A survey of non-starter lactic acid bacteria in traditional cheeses: Culture dependent identification and survival to simulated gastrointestinal transit. Int. Dairy J. 43: 42-50.

Rizzello, C. G., M. Calasso, D. Campanella, M. De Angelis and M. Gobbetti. 2014. Use of sourdough fermentation and mixture of wheat, chickpea, lentil and bean flours for enhancing the nutritional, texture and sensory characteristics of white bread. Int. J. Food Microbiol. 180: 78-87.

Rocha, J. M. and F. X. Malcata. 1999. On the microbiological profile of traditional portuguese sourdough. J. Food Protect. 62: 1416-1429.

Sakandar, H. A., R. Hussain, S. Kubow, F. A. Sadiq, W. Huang and M. Imran. 2019. Sourdough bread: A contemporary cereal fermented product. J. Food Process Preserv. 43: e13883.

Scarnato, L., C. Montanari, D. I. Serrazanetti, I. Aloisi, F. Balestra, S. Del Duca and R. Lanciotti. 2017. New bread formulation with improved rheological properties and longer shelf-life by the combined use of transglutaminase and sourdough. LWT. Food Sci. Technol. 81: 101-110.

Siezen, R. J., J. Kok, T. Abee and G. Schasfsma. (Eds.). 2002. Lactic Acid Bacteria: Genetics, Metabolism and Applications. Springer, Netherlands.

Singh, G., A. K. Verma and V. Kumar. 2015. Catalytic properties, functional attributes and industrial applications of β-glucosidases. 3 Biotech. 6: 3.

Tieking, M. and M. G. Gänzle. 2005. Exopolysaccharides from cereal-associated lactobacilli. Trends Food Sci.Technol. 16: 79-84.

Valmorri, S., R. Tofalo, L. Settanni, A. Corsetti and G. Suzzi. 2010. Yeast microbiota associated with spontaneous sourdough fermentations in the production of traditional wheat sourdough breads of the Abruzzo region (Italy). Antonie van Leeuwenhoek. 97: 119-129.

Yıldız, B., T. Ceyhan and A. Çakıcı. 2019. Sourdough yeasts in bread making: A review. 5: 33-46.